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Mycoflora Diversity and Fungal Contamination of Commercial Herbal Medicines Marketed in Jammu, Jammu and Kashmir (UT), India

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ABSTRACT

Keywords

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Herbal medicinal plants are widely used in traditional healthcare systems, but their safety and quality may be affected by microbial contamination during harvesting, processing, storage, and marketing. The present study was undertaken to investigate the fungal diversity associated with selected dried herbal medicinal plant samples. A total of ten herbal samples representing different plant parts, including rhizomes, leaves, stems, roots, and fruits, were collected from local herbal markets and analyzed for fungal contamination using the dilution plate technique. The fungal isolates were identified based on colony morphology and microscopic characteristics. The results revealed the presence of 17 fungal species belonging to several genera, including *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, *Rhizopus*, and *Mucor*. Among the investigated samples, *Terminalia belerica* fruits exhibited the highest fungal load and diversity, with a total colony count of 313 and a mean value of 18.41 ± 10.72 , whereas *Acorus calamus* rhizomes and *Azadirachta indica* leaves showed comparatively lower fungal contamination. The dominant fungal genera observed across the samples were *Aspergillus*, *Penicillium*, and *Fusarium*, which are commonly associated with stored plant materials and include several mycotoxin-producing species. The findings highlight the importance of proper post-harvest handling, hygienic processing, and suitable storage conditions to minimize fungal contamination and ensure the microbiological safety and quality of herbal medicines.

Introduction

Medicinal plants have been used since ancient times for the prevention and treatment of various human ailments and continue to play an important role in traditional healthcare systems worldwide. Plant-based medicines form the foundation of several traditional medical systems and remain an essential component of

healthcare, particularly in developing countries. According to the World Health Organization (WHO), nearly 70–80% of the global population relies on herbal medicines for primary healthcare, highlighting the importance of medicinal plants in modern and traditional medicine. The increasing global demand for herbal products is largely attributed to their perceived safety, therapeutic efficacy, cultural acceptance, and relatively

low cost compared with synthetic pharmaceuticals (Ekor, 2014; Singh *et al.*, 2024).

Despite their widespread use, herbal medicinal materials are highly susceptible to microbial contamination during different stages of production, including harvesting, drying, transportation, storage, processing, and marketing. Environmental conditions such as temperature, humidity, and poor hygienic practices significantly influence the growth and proliferation of microorganisms on dried plant materials. Among these contaminants, fungi represent one of the most important groups of microorganisms affecting herbal drugs. Fungal contamination not only leads to deterioration in the quality and shelf life of medicinal plant materials but may also result in the production of toxic secondary metabolites known as mycotoxins, which can pose serious health hazards to consumers (Bennett & Klich, 2003; Ałtyn & Twarużek, 2020).

Several fungal genera have been reported to contaminate medicinal plants and herbal drug preparations. Among them, species belonging to *Aspergillus*, *Penicillium*, and *Fusarium* are the most frequently encountered fungi in stored plant materials due to their ability to grow under conditions of low moisture and water activity (Pitt & Hocking, 2009). These fungi are widely distributed in nature and their spores are easily dispersed through air, soil, and dust, facilitating contamination of herbal plant materials during storage and handling. In addition, other fungi such as *Cladosporium*, *Rhizopus*, and *Mucor* are also commonly associated with medicinal plants and stored agricultural products (Samson *et al.*, 2010).

Mycotoxin-producing fungi are of particular concern because they can synthesize toxic compounds that may contaminate herbal products and pose significant health risks. Species such as *Aspergillus flavus*, *Aspergillus ochraceus*, and several *Fusarium* species are known to produce important mycotoxins including aflatoxins, ochratoxins, and fumonisins. These toxins are associated with a variety of adverse health effects including hepatotoxicity, nephrotoxicity, immunosuppression, and carcinogenicity (Bennett & Klich, 2003; Wei *et al.*, 2023). Consequently, the presence of toxigenic fungi in herbal medicines represents a major public health concern, particularly when contaminated plant materials are consumed over long periods (Kanabus *et al.*, 2025).

Medicinal plant materials are particularly vulnerable to fungal colonization because they contain nutrient-rich

organic compounds such as carbohydrates, proteins, and lipids, which provide favorable substrates for microbial growth. Furthermore, inadequate drying and improper storage conditions may increase moisture levels, thereby creating an ideal environment for fungal proliferation. Pitt and Hocking (2009) reported that storage fungi such as *Aspergillus* and *Penicillium* are capable of surviving and growing even under relatively dry conditions, making dried herbal products highly susceptible to contamination.

Several studies conducted worldwide have documented fungal contamination in herbal medicinal products. Chen *et al.*, (2020) reported that fungal communities associated with contaminated herbal medicines were dominated by species of *Aspergillus*, *Penicillium*, and *Fusarium*, which are commonly found in stored plant materials. Similarly, Ałtyn and Twarużek (2020) highlighted that improper storage and handling practices significantly increase the risk of fungal contamination and mycotoxin production in medicinal herbs. Recent investigations have also confirmed the presence of diverse toxigenic fungi in herbal medicines, emphasizing the importance of microbiological quality assessment (Hu *et al.*, 2024).

In India, where medicinal plants play a vital role in traditional healthcare systems such as Ayurveda and Unani medicine, several investigations have also reported fungal contamination in herbal drugs. Bhattacharya and Raha (2020) observed that medicinal plant materials stored under unfavorable environmental conditions are highly susceptible to fungal colonization. Sharma *et al.*, (2021) further reported that many herbal plant samples marketed in local markets may harbor fungal species capable of producing mycotoxins, thereby posing potential health risks to consumers.

More recently, Wei *et al.*, (2023) emphasized the occurrence of toxigenic fungi in herbal medicines and highlighted the importance of advanced detection techniques for monitoring fungal contamination. Similarly, Singh *et al.*, (2024) stressed the need for strict quality control and proper storage practices in herbal drug production to minimize microbial contamination and ensure product safety.

Considering the increasing global demand for herbal medicines and the potential health risks associated with fungal contamination, it is essential to assess the microbiological quality of medicinal plant materials. Therefore, the present study was undertaken to

investigate the fungal diversity associated with selected dried herbal medicinal plant samples and to evaluate the level of fungal contamination present in these materials.

Materials and Methods

Sample Collection

A total of ten dried herbal medicinal plant samples were collected from local herbal markets. The samples represented different plant parts commonly used in traditional medicine, including rhizomes, leaves, fruits, roots, and stems. The selected herbal samples included *Acorus calamus* (rhizome), *Azadirachta indica* (leaves), *Curcuma longa* (rhizome), *Glycyrrhiza glabra* (stem), *Ocimum sanctum* (leaves), *Phyllanthus emblica* (fruit), *Saussurea costus* (root), *Terminalia belerica* (fruit), *Terminalia chebula* (fruit), and *Viola odorata* (stem and leaves). Each sample was collected in sterile polyethylene bags and transported to the laboratory for further microbiological analysis. The samples were stored in dry conditions at room temperature until processing.

Sample Preparation

All samples were processed under aseptic conditions in the laboratory. Approximately 1 g of dried plant material from each sample was finely crushed using a sterile mortar and pestle. The powdered sample was transferred into 9 ml of sterile distilled water to obtain a 10^{-1} dilution. The mixture was thoroughly shaken to ensure proper suspension of fungal spores and other microorganisms present in the sample.

Serial Dilution

Serial dilutions were prepared by transferring 1 ml of the initial suspension into 9 ml of sterile distilled water to obtain successive dilutions up to 10^{-3} or 10^{-4} , depending on the level of contamination in the sample. The dilution technique was used to obtain countable fungal colonies during plating.

Isolation of Fungi

Fungal isolation was carried out using the dilution plate technique. From the prepared dilutions, 0.1 ml of the suspension was spread evenly onto sterile Potato Dextrose Agar (PDA) plates supplemented with

antibiotics to inhibit bacterial growth. The inoculated plates were incubated at $25 \pm 2^\circ\text{C}$ for 5–7 days. After incubation, fungal colonies appearing on the agar plates were observed and counted.

Enumeration of Fungal Colonies

The number of fungal colonies developing on each plate was counted and expressed as colony forming units per gram (CFU/g) of the sample.

The CFU was calculated using the following formula:

$$CFU/g = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume of inoculum plated (ml)}}$$

Where:

- Number of colonies = colonies counted on the agar plate
- Dilution factor = reciprocal of the dilution used
- Volume plated = volume of diluted sample inoculated onto the plate (0.1 ml)

The colony counts were recorded for each sample, and the mean value and standard deviation (Mean \pm SD) were calculated to determine the level of fungal contamination among the analyzed herbal samples.

Identification of Fungal Isolates

Fungal isolates were identified based on macroscopic and microscopic characteristics. Colony morphology such as color, texture, growth pattern, and margin was examined on PDA plates. Microscopic examination was carried out using lactophenol cotton blue staining, which allowed observation of fungal structures such as hyphae, conidiophores, conidia, and sporangia under a compound microscope. Identification of fungal species was performed using standard mycological identification manuals.

Results and Discussion

Characteristics of Herbal Samples (Table 1)

The details of the herbal medicinal plant samples analyzed in the present study are presented in Table 1. A total of four commercially available herbal medicines were selected, including *Acorus calamus*, *Azadirachta indica*, *Glycyrrhiza glabra*, and *Terminalia belerica*.

These plants represent different plant parts commonly used in traditional medicine, such as rhizomes, leaves, stems, and fruits.

All the samples were obtained in whole dried form, which is the common form in which herbal materials are marketed and stored. The variation in plant parts analyzed in the study provided a basis for evaluating fungal contamination in different herbal drug materials.

The information summarized in Table 1 describes the basic characteristics of the samples used for the mycological investigation.

Fungal Diversity and Colony Distribution (Table 2)

The mycological analysis of selected herbal samples revealed the presence of 17 fungal species belonging to several genera, including *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, *Rhizopus*, *Mucor*, *Botrytis*, *Chaetomium*, *Geotrichum*, and *Trichothecium* (Table 2). The fungal isolates showed considerable variation in colony numbers, CFU/g values, and frequency percentages among the different herbal materials.

Among the investigated samples, *Terminalia belerica* fruits exhibited the highest fungal diversity and colony abundance. The dominant species in this sample was *Penicillium expansum*, which recorded the highest colony count of 42 colonies corresponding to 4.2×10^5 CFU/g, followed by *Penicillium chrysogenum* (34 colonies; 3.4×10^5 CFU/g) and *Fusarium moniliforme* (30 colonies; 3.0×10^5 CFU/g). Other frequently occurring fungi included *Aspergillus niger*, *Aspergillus ochraceus*, and *Fusarium oxysporum*.

In the dried rhizome of *Acorus calamus*, ten fungal species were isolated. The most dominant fungus was *Penicillium chrysogenum* with 23 colonies (2.3×10^5 CFU/g) and a frequency of 15.54%, followed by *Aspergillus niger* (21 colonies; 14.19%) and *Fusarium moniliforme* (18 colonies; 12.16%). Moderate frequencies were observed for *Cladosporium oxysporum*, *Penicillium expansum*, and *Rhizopus stolonifer*.

The dried leaves of *Azadirachta indica* also showed moderate fungal contamination, where *Penicillium chrysogenum* was the most dominant species with 30 colonies (3.0×10^5 CFU/g) and a frequency of 20.55%.

Other commonly occurring fungi included *Fusarium moniliforme*, *Penicillium expansum*, *Fusarium oxysporum*, and *Aspergillus niger*.

In the dried stem of *Glycyrrhiza glabra*, nine fungal species were recorded. The highest colony count was observed for *Fusarium moniliforme*, which showed 31 colonies corresponding to 3.1×10^5 CFU/g and a frequency of 17.51%. Other prominent fungi included *Aspergillus flavus*, *Fusarium oxysporum*, and *Aspergillus ochraceus*.

Overall, the genera *Aspergillus*, *Penicillium*, and *Fusarium* were the most frequently encountered fungi across the analyzed herbal samples, indicating their common occurrence in dried plant materials.

Statistical Summary of Fungal Colonies (Table 3)

The statistical analysis of fungal colonies revealed noticeable differences in total colony counts and mean colony values among the herbal samples (Table 3).

The highest fungal load was observed in *Terminalia belerica*, which recorded a total of 313 colonies with a mean value of 18.41 ± 10.72 colonies. This indicates comparatively higher fungal abundance and variability in the fruit samples.

In contrast, *Glycyrrhiza glabra* showed a moderate fungal load with 177 total colonies and a mean value of 10.41 ± 10.52 colonies.

The dried rhizome of *Acorus calamus* and the leaves of *Azadirachta indica* exhibited relatively lower fungal counts, recording 148 colonies (8.71 ± 8.59) and 146 colonies (8.59 ± 9.36) respectively.

The comparatively higher fungal colonization observed in fruit samples suggests that nutrient composition, surface structure, and storage conditions may significantly influence fungal growth in dried herbal materials.

The present investigation revealed that dried herbal medicinal samples harbor a diverse community of fungal contaminants. Such contamination may occur during harvesting, drying, transportation, storage, and marketing of herbal products, where airborne fungal spores can easily colonize plant materials.

Table.1 List of herbal medicines analyzed and sample characteristics

S. no	Herbal name	Plant part used	Form (whole/powdered)
1	<i>Acorus calamus</i>	Rhizome	whole dried
2	<i>Azadiracta indica</i>	Leaves	whole dried
3	<i>Glycyrrhiza glabra</i>	Stem	Whole dried
4	<i>Terminalia belrica</i>	Fruit	Whole dried

Table.2 Colony forming units (CFU/g) and frequency (%) of fungal species isolated from different dried herbal samples at 10⁻³ dilution.

S. no	Fungal species	<i>Acorus calamus</i> (dried rhizome)			<i>Azadiracta indica</i> (dried leaves)			<i>Glycyrrhiza glabra</i> (dried stem)			<i>Terminalia belerica</i> (dried fruits)		
		no. of colonies	CFU/g (0.1ml)	Freq %	no. of colonies	CFU/g (0.1ml)	Freq %	no. of colonies	CFU/g (0.1ml)	Freq %	no. of colonies	CFU/g (0.1ml)	Freq %
1	<i>A. niger</i>	21	2.1×10 ⁵	14.19	12	1.2×10 ⁵	8.22	18	1.8×10 ⁵	10.17	28	2.8×10 ⁵	8.95
2	<i>Aspergillus flavus</i>	9	9×10 ⁴	6.08	6	6×10 ⁴	4.11	23	2.3×10 ⁵	12.99	19	1.9×10 ⁵	6.07
3	<i>Aspergillus ochraceus</i>	0	0	0	6	6×10 ⁴	4.11	21	2.1×10 ⁵	11.86	26	2.6×10 ⁵	8.31
4	<i>Aspergillus terreus</i>	0	0	0	9	9×10 ⁴	6.16	16	1.6×10 ⁵	9.04	14	1.4×10 ⁵	4.47
5	<i>Botrytis cinerea</i>	0	0	0	0	0	0	0	0	0	9	9×10 ⁴	2.88
6	<i>Chaetomium globosum</i>	0	0	0	0	0	0	0	0	0	7	7×10 ⁴	2.24
7	<i>Cladosporium oxysporum</i>	17	1.7×10 ⁵	11.49	12	1.2×10 ⁵	8.22	10	1.0×10 ⁵	5.65	20	2.0×10 ⁵	6.39
8	<i>Fusarium moniliforme</i>	18	1.8×10 ⁵	12.16	21	2.1×10 ⁵	14.38	31	3.1×10 ⁵	17.51	30	3.0×10 ⁵	9.58
9	<i>Fusarium oxysporum</i>	12	1.2×10 ⁵	8.11	17	1.7×10 ⁵	11.64	22	2.2×10 ⁵	12.43	23	2.3×10 ⁵	7.35
10	<i>Fusarium sporotrichioides</i>	0	0	0	0	0	0	0	0	0	11	1.1×10 ⁵	3.51
11	<i>Geotrichum candidum</i>	0	0	0	0	0	0	0	0	0	4	4×10 ⁴	1.28
12	<i>Mucor sp.</i>	9	9×10 ⁴	6.08	0	0	0	0	0	0	14	1.4×10 ⁵	4.47
13	<i>Penicillium chrysogenum</i>	23	2.3×10 ⁵	15.54	30	3.0×10 ⁵	20.55	19	1.9×10 ⁵	10.73	34	3.4×10 ⁵	10.86
14	<i>Penicillium expansum</i>	17	1.7×10 ⁵	11.49	21	2.1×10 ⁵	14.38	6	6×10 ⁴	3.39	42	4.2×10 ⁵	13.42
15	<i>Penicillium purpurogenum</i>	6	6×10 ⁴	4.05	0	0	0	0	0	0	10	1.0×10 ⁵	3.19
16	<i>Rhizopus stolonifer</i>	16	1.6×10 ⁵	10.81	12	1.2×10 ⁵	8.22	11	1.1×10 ⁵	6.21	15	1.5×10 ⁵	4.79
17	<i>Trichothecium roseum</i>	0	0	0	0	0	0	0	0	0	7	7×10 ⁴	2.24

Table.3 Statistical summary (total colonies and mean ± SD) of fungi isolated from different dried herbal samples.

Parameter	<i>Acorus calamus</i>	<i>Azadiracta indica</i>	<i>Glycyrrhiza glabra</i>	<i>Terminalia belerica</i>
Total colonies	148	146	177	313
Mean ± SD (colonies)	8.71 ± 8.59	8.59 ± 9.36	10.41 ± 10.52	18.41 ± 10.72

Figure.1 Distribution of Fungal colonies in Herbal Medicinal Samples

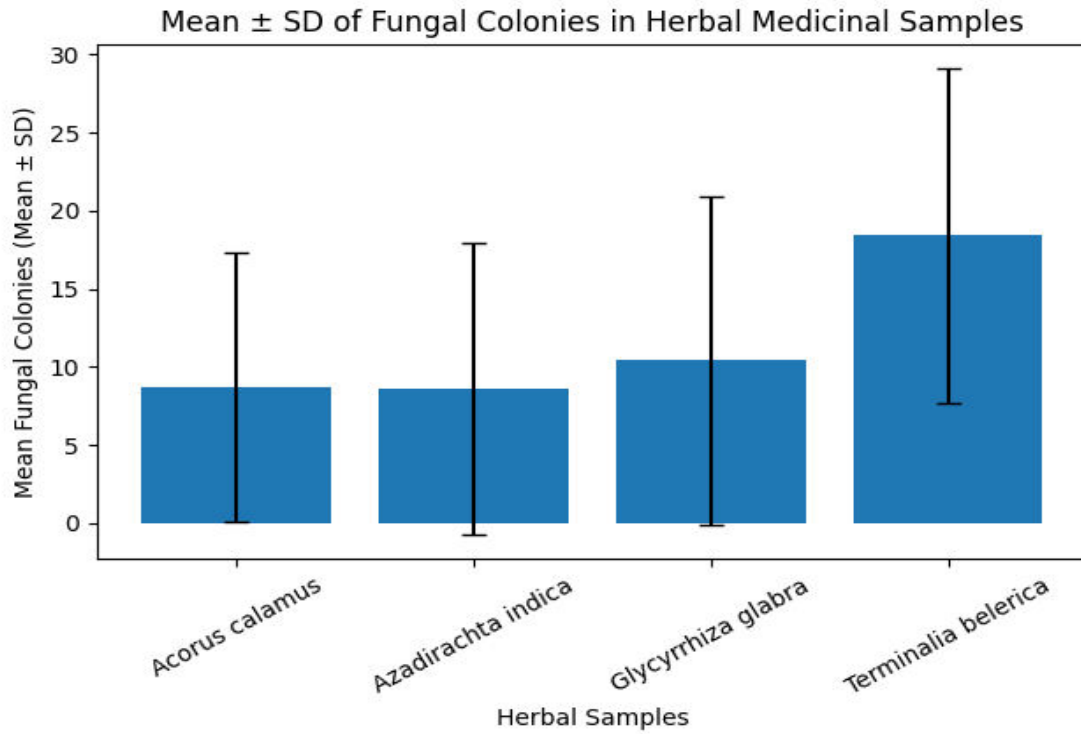
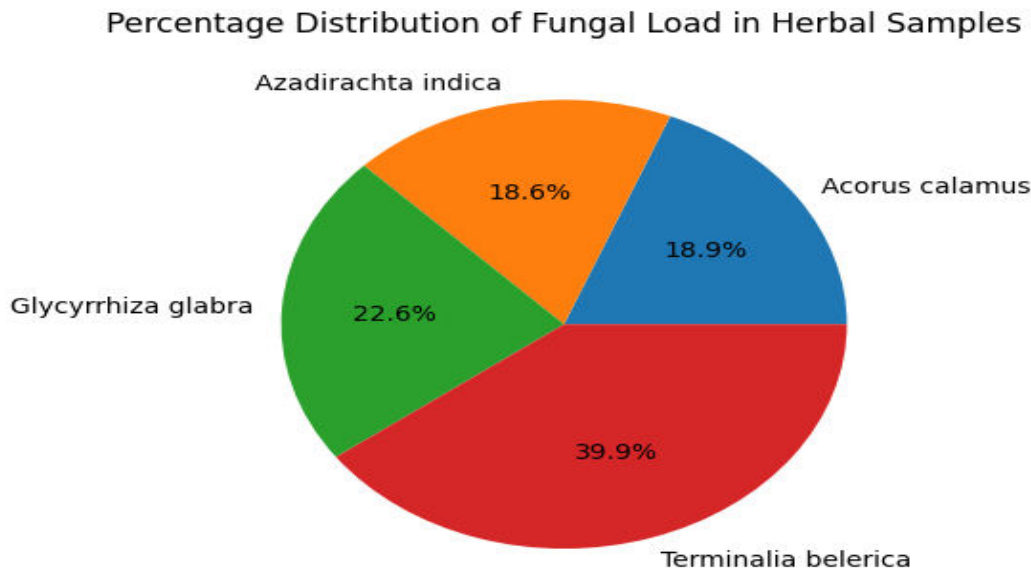


Figure.2 Percentage distribution of Fungal load in Herbal samples



Dried plant substrates provide favorable conditions for fungal growth because of the presence of organic nutrients and suitable microenvironmental conditions (Pitt & Hocking, 2009; Altyn & Twarużek, 2020).

In the present study, 17 fungal species belonging to several genera, including *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, *Rhizopus*, and *Mucor*, were isolated from the analyzed herbal samples. Similar

fungal genera have frequently been reported from medicinal plants and herbal drugs in previous studies (Samson *et al.*, 2010; Chen *et al.*, 2020). Investigations on herbal medicines have shown that *Aspergillus*, *Penicillium*, and *Fusarium* species are among the most common fungal contaminants associated with dried plant materials due to their ability to survive under low moisture conditions and adapt to storage environments (Wei *et al.*, 2023).

Among the analyzed samples, *Terminalia bellerica* fruits exhibited the highest fungal diversity and colony abundance, which was supported by both colony counts and statistical analysis. Fruit-based herbal materials are generally more susceptible to fungal colonization because they contain higher levels of carbohydrates and other nutrients that support fungal growth during storage. Similar observations have been reported in studies on fungal contamination of medicinal plants and herbal products (Chen *et al.*, 2020; Singh *et al.*, 2024).

In contrast, *Acorus calamus* rhizomes and *Azadirachta indica* leaves showed comparatively lower fungal loads. The reduced fungal growth observed in these samples may be associated with the presence of natural antimicrobial and antifungal compounds present in these plants. *Azadirachta indica*, in particular, is well known for its antifungal properties due to bioactive constituents such as azadirachtin and nimbin (Subapriya & Nagini, 2005). Similar findings have been reported in previous studies that documented reduced microbial contamination in medicinal plants containing strong bioactive phytochemicals (Bhattacharya & Raha, 2020; Sharma *et al.*, 2021).

The predominance of fungi belonging to the genera *Aspergillus*, *Penicillium*, and *Fusarium* observed in the present study is consistent with earlier reports on fungal contamination of stored herbal drugs and medicinal plant materials (Raper & Fennell, 1965; Pitt & Hocking, 2009). These fungi are known to thrive in dried plant substrates because they are capable of growing under low water activity and storage conditions (Samson *et al.*, 2010).

The presence of species such as *Aspergillus flavus*, *Aspergillus ochraceus*, and *Fusarium moniliforme* is particularly significant because these fungi are known producers of mycotoxins, including aflatoxins, ochratoxins, and fumonisins. These toxic metabolites may pose serious health risks if contaminated herbal

materials are consumed or used in medicinal preparations (Bennett & Klich, 2003).

Recent studies have also reported the occurrence of mycotoxin-producing fungi in herbal medicines and emphasized the need for strict quality control and microbial monitoring of medicinal plant products (Kumar *et al.*, 2023; Kanabus *et al.*, 2025).

The statistical analysis of fungal colonies revealed variation in colony numbers and mean colony counts among the investigated herbal samples. The relatively high standard deviation values observed in the study suggest heterogeneous distribution of fungal species, which may be influenced by environmental factors such as temperature, humidity, storage duration, and handling practices. Similar variability in fungal contamination levels among different herbal products has been reported in previous studies (Agarwal *et al.*, 2021; Chaudhary *et al.*, 2022).

Overall, the findings of the present study indicate that dried herbal medicinal products are susceptible to fungal contamination, which may affect their quality, shelf life, and safety. Therefore, proper post-harvest processing, hygienic handling, controlled drying techniques, and appropriate storage conditions are essential to reduce fungal contamination and ensure the microbiological quality of herbal medicines.

In conclusion, the present study highlights the occurrence of diverse fungal contaminants associated with dried herbal medicinal materials. A total of 17 fungal species belonging to several genera, including *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, *Rhizopus*, and *Mucor* were isolated from the analyzed samples, indicating that dried plant products can act as reservoirs for various fungal microorganisms. Among the investigated samples, *Terminalia bellerica* fruits exhibited the highest fungal load and diversity, whereas *Acorus calamus* rhizomes and *Azadirachta indica* leaves showed comparatively lower fungal contamination.

The predominance of fungal genera such as *Aspergillus*, *Penicillium*, and *Fusarium*, which include several mycotoxin-producing species, is of particular concern because these fungi may affect the quality, safety, and therapeutic efficacy of herbal medicines. The variability in colony counts among different plant materials suggests that plant composition, moisture content, environmental exposure, and storage conditions play significant roles in determining fungal colonization.

These findings emphasize the importance of proper harvesting practices, hygienic handling, controlled drying techniques, and appropriate storage conditions to minimize fungal contamination in herbal products. Regular microbiological monitoring and quality control measures should be implemented to ensure the safety and efficacy of medicinal plant materials.

Overall, the present investigation contributes valuable information on the mycological quality of commonly used herbal medicines and highlights the need for strict quality standards and improved post-harvest management practices in the herbal drug industry to protect consumer health and maintain the therapeutic value of medicinal plants.

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Author Contributions

Yash Paul Singh: Investigation, formal analysis, writing—original draft. Mala Bhasin: Validation, methodology, writing—reviewing. Harjeet Kour Sodhi:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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